ADVANCES IN MICROBIAL ECOLOGY

Edited by K. C. Marshall

Volume 9

Advances in MICROBIAL ECOLOGY

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Advances in MICROBIAL ECOLOGY

Volume 9

Edited by

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Preface

Advances in Microbial Ecology was established by the International Committee on Microbial Ecology (ICOME) to provide a means for in-depth, critical, and even provocative reviews to emphasize current trends in the rapidly expanding area of microbial ecology. *Advances in Microbial Ecology* is now recognized as a major source of information and inspiration both for practicing and for prospective microbial ecologists. The majority of reviews published in *Advances* have been prepared by leaders in particular areas following invitations provided by the Editorial Board. Although the Board intends to continue its policy of soliciting reviews, individual microbial ecologists are encouraged to submit outlines of unsolicited contributions to any member of the Editorial Board for consideration for inclusion in *Advances.*

Volume 9 of *Advances in Microbial Ecology* covers a particularly broad range of topics related to microbial ecology. The potential for applying ribosomal RNA sequence analysis for the definition of natural microbial populations is considered by N. R. Pace, D. A. Stahl, D. J. Lane, and G. J. Olsen. Other reviews on techniques include the application of microelectrode technology to microbial ecosystems by N. P. Revsbech and B. B. Jørgensen and the use of rates of nucleic acid synthesis to determine bacterial growth rates in natural aquatic habitats by D. J. W. Moriarty. The contribution by T. Fenchel discusses the ecology of heterotrophic microflagellates. J. H. Andrews and R. F. Harris present the concept of *r*- and *K*-selection and its relevance to microbial ecology. Recent studies on the rhizosphere are discussed by J. M. Whipps and J. M. Lynch in the context of the influence of the rhizosphere on crop production. M. Rosenberg and S. Kjelleberg consider the importance of

hydrophobic interactions in the behavior of bacteria at various interfaces. Finally, J. G. Jones reviews the role of bacteria in the cycling of iron in freshwater ecosystems.

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Hydrophobic Interactions: Role in Bacterial Adhesion

MEL ROSENBERG and STAFFAN KJELLEBERG

1. Introduction

The last decade has seen a dramatic rise in scientific interest in the field of bacterial adhesion and related subjects. Much of this interest has been directed in search of "specific interactions" between bacterium and substratum, such as those that are inhibited by specific sugar moieties. A second avenue of research has been the study of the role of less specific, hydrophobic interactions. Since it appears that this second avenue of research is being increasingly followed, reviews dealing with bacterial hydrophobicity and adhesion should be of use. The aims of the present chapter are to (1) present some relevant aspects of hydrophobic interactions; (2) describe the methodology available for measurements related to bacterial cell-surface hydrophobicity and the parameters they may measure; (3) discuss investigations dealing with surface components that promote or reduce bacterial hydrophobicity; and (4) survey studies related to the role of hydrophobic interactions in mediating bacterial adhesion to interfaces, traversing areas of environmental and medical interest.

The importance of bacterial hydrophobicity in adhesion to liquid–liquid interfaces and the possible relationship between cell-surface hydro-

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phobicity and phagocytosis were reported over 60 years ago by Mudd and Mudd (1924a,b). These authors observed that certain microorganisms (e.g., *Erythrobacillus prodigiosus,* currently known as *Serratia marcescens*) tend to partition at the water–oil interface, and provided a theoretical definition of this phenomenon in terms of bacteria–oil, bacteria–water, and oil–water interfacial energies. Reed and Rice (1931) made an unsuccessful attempt to quantify bacterial partitioning at the oil–water interface, presumably because of their limited choice of bacterial strains. However, these authors were able to show that certain bacteria (e.g., mycobacteria) are able to pass from the bulk aqueous phase to the bulk oil phase upon vigorous mixing.

In the subsequent four decades, relatively little scientific interest was directed toward the study of bacterial hydrophobicity, with notable exceptions in the work of Dyar (1948) and Hill *et al.* (1963) and studies on bacterial interactions at the air–water interface (e.g., Boyles and Lincoln, 1958). Interest in bacterial hydrophobicity and adhesion was renewed with the work of Marshall and coworkers (Marshall and Cruickshank, 1973; Marshall *et al.,* 1975) and van Oss and colleagues [for a review see van Oss (1978)] in the early 1970s.

During the past decade, a variety of techniques for measuring bacterial hydrophobicity have been proposed, accompanied by a surge in research, as can be discerned from Fig. 1.

Hydrophobic interactions have been implicated in a wide array of adhesion phenomena, including partitioning at the oil–water (M. Rosenberg, 1984a) and air–water (Kjelleberg, 1985) interfaces and adhesion to plastics (Fletcher, 1976; Fletcher and Marshall, 1982b), epithelial cells (M. Rosenberg *et al.,* 1981; E. Rosenberg, *et al.,* 1983a), phagocytes (van Oss, 1978), teeth (M. Rosenberg *et al.,* 1983a,c; Nesbitt *et al.,* 1982), sub-

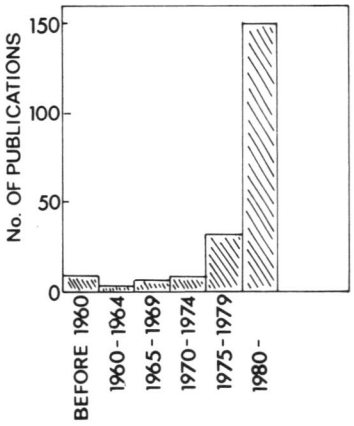

Figure 1. Hydrophobicity- and adhesion-related papers as a function of publication period. As an indication of interest in hydrophobicity and adhesion, the distribution of the publications referred to in this review into publication periods is illustrated. A burst in interest over the past decade is evident.

merged aquatic surfaces (Fattom and Shilo, 1984), sulfur granules (Bryant *et al.*, 1984), mineral surfaces (Stenstrom and Kjelleberg, 1985), and to one another (Stanley and Rose, 1967; Moore and Marshall, 1981; Faris *et al.*, 1983). In several cases, surface components contributing to bacterial hydrophobicity have been identified. Bacterial hydrophobicity may also be an important factor in other adhesion-related phenomena, such as gliding motility (Dworkin *et al.*, 1983; Humphrey *et al.*, 1979; R. Wolkin and J. L. Pate, in preparation) and colony spreading (Gibbons *et al.*, 1983).

Despite the apparent importance of hydrophobic interactions in participating in such a wide range of phenomena, the term "bacterial hydrophobicity" has no unequivocal definition, nor a definitive scale of values. This is not surprising in light of current disagreements among scientists on the precise nature of hydrophobic interactions (e.g., Hildebrand, 1979; Israelachvili and Pashley, 1984) and the lack of consensus on a definitive method for measuring bacterial hydrophobicity (see Section 3). Thus, the literature is replete with confusing terminologies ("pronounced" versus "slight" cell-surface hydrophobicity) and results from various laboratories that cannot be compared with one another.

Are we then to conclude that the plurarity of methods for measuring bacterial hydrophobicity is detrimental? Actually, the opposite is probably the case. The nonuniformity of the bacterial cell surface confounds attempts at a universal criterion: a bacterium that is hydrophilic all over, save for a hydrophobic tip (Marshall and Cruickshank, 1973), may be hydrophilic in one assay and hydrophobic in another. Moreover, the various techniques have given researchers flexibility in approaching various adhesion problems.

Cell-surface hydrophobicity is by no means a subject of interest restricted to bacteriology, and has been implicated in a wide variety of eucaryotic cell interactions and viral adhesion as well. Although such phenomena are beyond the scope of this review, we have listed some examples in Table I.

2. The Hydrophobic Effect

The general concept of hydrophobic interactions in adhesion and other biological systems has been extensively reviewed in recent years (Magnusson, 1980, 1982; Rutter, 1980; Edebo *et al.*, 1980; Ochoa, 1978). Several alternate approaches to explaining hydrophobic interactions have been taken by various investigators. The present section is thus an attempt to illustrate several viewpoints that have gained some consensus.

One definition for hydrophobic interactions involves the free energy

Table I. Hydrophobicity and Adhesion in Nonbacterial Systems

Phenomenon	Reference
Adhesion of poliovirus to membrane filters	Shields and Farrah (1983)
Foam separation of virus	Morrow (1969)
Adhesion of fungal spores *(Colletotrichum lindemuthianum)* to polystyrene and plant surfaces	Young and Kauss (1984)
Adhesion and growth of yeast on hydrocarbons	Nakahara *et al.* (1981), Miura *et al.* (1977), McLee and Davies (1972), Kappeli and Fiechter (1976)
Sexual interactions of *Paramecium caudatum*	Kitamura (1984)
Invasion of *Plasmodium falciparum* into erythrocytes	Breuer *et al.* (1984)
Adhesion to connective tissue	Wang (1974)
Adhesion properties of tissue culture cells	Malmqvist *et al.* (1984), Reuveny *et al.* (1983)
Adhesion of tumor cells	Hills (1984b)
Adhesion and surface properties of erythrocytes	Todd and Gingell (1980), Neumann *et al.* (1983)
Partitioning of sake yeast at the air–water interface	Ouchi and Akiyama (1971)

change ΔG associated with the process of bringing two entities from infinite separation to distances of the order of magnitude of molecular dimensions (Ben-Naim, 1977). In our case, this free energy change relates to the layering of water molecules at cell and substratum surfaces. According to Ben-Naim (1977), a nonpolar moiety introduced into an aqueous phase will be surrounded by water molecules whose structure is greater than that of molecules in the bulk aqueous phase. Such structured water layer(s) are associated with a decrease in entropy ($\Delta S < 0$) related to the increased order of the system. Thus, in order to solubilize nonpolar solutes, a "hole" must be created in the water structure of the bulk phase; the ensuing breaking of bonds between water molecules demands an input of energy. The formation of new hydrogen bonds in such a process leads to a negative but very small enthalpy change ΔH. Thus, the energy input required to carry out this process may be attributed to the negative entropy change.

What occurs when two hydrophobic surfaces covered by structured water molecules approach one another? In such a case, layers of structured water molecules are released into the bulk aqueous phase from the contacting surfaces. This situation is illustrated schematically in Fig. 2. The freeing of such molecules brings about the reverse situation to that described above: a decrease in free energy due to reduction of the overall nonpolar–water interface by the transfer of water molecules to the less

ordered bulk water state (i.e., increase in ΔS). This premise, based on the behavior of water molecules surrounding apolar "cavities" (Arakawa *et al.,* 1979), rests on experimental evidence suggesting that contributions from formation of surface–surface and breaking of surface–water interactions can largely be neglected. The free energy for hydrophobic interactions thus has its largest contribution from the cavity effect, i.e., the entropy function.

Several properties of hydrophobic interactions can serve as criteria for their participation in adhesion of two surfaces. Hydrophobic interactions generally increase with ionic strength due to the suppression of electrostatic interactions (Ochoa, 1978). To a certain point, hydrophobic interactions increase with increasing temperature (Ben-Naim, 1977). Temperature may, however, also provoke increased solvation and decreased surface tension, which counteract the hydrophobic interactions. Hydrophobic interactions are also expected to increase with increasing apolar nature of one or both surfaces under consideration. Hydrophobic interactions are also enhanced by salting-out ions, according to the Hofmeister series (Lindahl *et al.,* 1981). At high concentrations of salting-out ions, the solubility of the solute is adversely affected by decreasing availability of water molecules in the bulk and increasing surface tension of water, resulting in an increase in hydrophobic interactions (Tanford, 1973). The increase in surface tension of water in the presence of salting-out agents may be indicative of increased water structure at low-energy (hydrophobic) interfaces (Tanford, 1973); freeing of increasingly structured water upon the approach of two hydrophobic surfaces, as discussed above, would, in turn, be increasingly favored. The effect of structure-breaking (chaotropic) ions may be viewed as the opposite case; the new ordering of water molecules that they induce prevents them from undergoing further positive entropy changes (Ochoa, 1978).

The above descriptions of hydrophobic interactions are derived from considerations of the close approach of extremely apolar moieties, such as two molecules of alkane. The role of hydrophobic interactions in bacterial adhesion is obviously far more complicated. The term "hydropho-

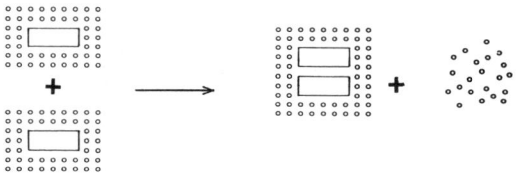

Figure 2. Aggregation of two nonpolar solutes due to hydrophobic interaction. When two separated polar solutes (represented by rectangles, left) covered by ordered layers of water molecules (represented by circles) interact, water molecules are released into the bulk aqueous phase (right). This interaction is accompanied by an increase in ΔS and a smaller increase in ΔH.

bic bacteria" is probably a misnomer: the great majority of bacteria referred to in this chapter are wetted by water and are readily suspended in aqueous medium due to polar, hydrophilic moieties, which abound on the bacterial cell surface. In fact, the ability of certain bacteria to adhere to oil droplets and prevent their coalescence (Rosenberg *et al.*, 1980a) is akin to oil emulsification by amphipathic molecules, which interact with both the water and oil phases. Similarly, the surfaces to which bacteria adhere often contain both polar and nonpolar constituents. The degree of each will influence the extent and structure of the proximal water molecule layers and thus determine the energetic favorability of adhesion, as previously discussed. Thus, it is the interplay between hydrophilic and hydrophobic surface components that determines the overall contribution of hydrophobic interactions to adhesion processes.

The outcome of an adhesion process can be predicted by surface thermodynamics (i.e., the the interfacial tensions between the adhering surfaces), as compared with that of each individual surface with the aqueous medium. Such interfacial tensions are themselves a reflection of the free energy interrelationships between the surfaces in question and the water molecules surrounding them. Development of such a model with respect to bacterial adhesion can be found in Absolom *et al.* (1983). These authors obtained two sets of experimental results; one set consisted of the interfacial tensions of the cells themselves; the second set consisted of adhesion measurements of the cells to surfaces of varying interfacial tensions in the presence of varying concentrations of surfactant (dimethylsulfoxide, DMSO). According to the concept put forward by these investigators, adhesion is independent of substratum surface energy when the surface energies of bacterium and suspending medium are equal. The results obtained for the surface energies of the bacteria tested based on their adhesion correlated well with the surface energies calculated from contact angle measurements. As predicted by their model, adhesion to hydrophilic surfaces was more pronounced only when the bacterial surface tension was larger than that of the surrounding aqueous medium; conversely, adhesion to increasingly hydrophobic surfaces occurred when the surface tension of the aqueous phase was higher than that of the bacterial cells. In many ecosystems, it is the second situation that prevails. In all experiments, moreover, adhesion was highest for the most hydrophobic bacteria for every aqueous surface tension studied.

3. Methods for Determining Cell-Surface Hydrophobicity

The methodology for studying the cell-surface hydrophobicity of bacterial cells can be divided into several groups, according to the type of

parameters measured: (1) methods based on adhesion of cells to solid surfaces, at interfaces, or to one another; (2) methods based on the partitioning of hydrophobic molecules between the cells and surrounding medium; (3) methods based on partitioning of cells between two immiscible liquid phases; (4) techniques based upon properties of cell layers; and (5) indirect methods, such as those based on the inhibition of adhesion by agents that interfere with hydrophobic interactions.

Some general factors pertain to most methods currently employed: (1) all materials should be scrupulously clean and free of surfactant contaminants; (2) growth conditions and preparation of bacterial cells should be carefully monitored; and (3) duration of the assay should be minimal, to prevent release of surface components that may have an inhibitory effect, e.g., by "conditioning" of the attachment surface (Fletcher and Marshall, 1982b), or alternatively, synthesis of cell-surface components that alter surface properties.

3.1. Cell-Surface Hydrophobicity Methods Based on Adhesion

In studies related to the role of hydrophobic interactions in bacterial adhesion, methods that are themselves based on adhesion are sometimes the most pertinent. It should be reemphasized that in cases in which adhesion is mediated by a small portion of the bacterial cell, e.g., in partitioning of *Hyphomicrobium, Flexibacter,* and *Rhizobium* cells at the oil–water interface (Marshall and Cruickshank, 1973, Marshall *et al.,* 1975), the surface properties of the large majority of the cell surface make only a small contribution.

3.1.1. Hydrophobic Interaction Chromatography (HIC)

This method, originally developed for protein separation (Hjertén *et al.,* 1974), has found widespread use in many studies of bacterial hydrophobicity. In this technique, aqueous suspensions of Sepharose beads with covalently bound hydrophobic moieties (e.g., phenyl or octyl groups) are usually packed into small columns and the bacterial suspension is subsequently applied. Retention may be determined by various means, such as turbidimetric readings or measurement of colony-forming units (Smyth *et al.,* 1978) or radioactivity (Hermansson *et al.,* 1982). In many cases, salting-out agents are added to promote adhesion to the gel (Smyth *et al.,* 1978). In some cases, adherent cells can be desorbed by lowering the ionic strength of the eluent or by adding detergent (Magnusson *et al.,* 1979a). Since it has been demonstrated that bacteria can become nonspecifically trapped within the gel matrix or between the gel and column (Olsson and Westergren, 1982), the assay is preferably performed by mixing of the beads with the bacterial cells, followed by sep-

aration of free from bound cells. Moreover, controls should be performed with the corresponding untreated Sepharose beads themselves, as in some cases bacteria may bind to them as well.

3.1.2. Bacterial Adhesion to Hydrocarbons (BATH)

As already mentioned, bacterial partitioning at the oil–water interface has been studied by various investigators since 1924 (Mudd and Mudd, 1924a,b; Marshall and Cruickshank, 1973; Marshall *et al.*, 1975), including many researchers in the field of petroleum microbiology (e.g., Neufeld *et al.*, 1980; Kennedy *et al.*, 1975; Kirschner-Zilber *et al.*, 1980, Nakahara *et al.*, 1981). In 1980, bacterial adhesion to hydrocarbons (BATH) was suggested as a simple and convenient assay for measuring bacterial hydrophobicity (M. Rosenberg *et al.*, 1980a). In this test, washed bacterial suspensions are vortexed under controlled conditions with an aliphatic (e.g., hexadecane) or aromatic (e. g., xylene) liquid hydrocarbon. In the case of adhesion, bacteria bind to the oil droplets and rise with them following the mixing procedure, bringing about a concomitant drop in the cell density of the lower bulk aqueous phase. This technique and various modifications have been used to study bacterial hydrophobicity in a wide variety of bacterial species and cell mixtures as a function of growth conditions and other parameters (M. Rosenberg, 1984a). The basic technique has also enabled enrichment of hydrophobic mutants in *Acinetobacter calcoaceticus* (M. Rosenberg and Rosenberg, 1981), *Serratia marcescens* (M. Rosenberg, 1984c), and *Streptococcus sanguis* (Gibbons *et al.*, 1983).

One of the main limitations of the method as employed thus far is its semiquantitative nature. A quantitative approach based on the kinetics of adhesion to hexadecane has recently been employed (D. Lichtenstein, M. Rosenberg, N. Sharfman, and I. Ofek, in preparation).

Results obtained using BATH usually correlate with those obtained using other techniques (M. Rosenberg, 1984a). An interesting anomaly in this regard is that enterobacterial strains, which appear to exhibit cell-surface hydrophobicity with other methods, adhere poorly or not at all to hexadecane. However, it was recently shown that adhesion of a rough *Escherichia coli* strain to hexadecane was promoted from 0 to 76% by adding ammonium sulfate to the bacterial suspension (M. Rosenberg, 1984b). This finding suggests that many enterobacterial strains purported to possess pronounced cell-surface hydrophobicity are actually much less hydrophobic than strains that adhere to hydrocarbons in the absence of salting-out agents, e.g., *Streptococcus pyogenes, Acinetobacter calcoaceticus,* and *Serratia marcescens.*

Several points concerning the BATH assay require further clarifica-

tion: (1) Might the vortexing procedure in the presence of hydrocarbons extract hydrophobic cell-surface components into the hydrocarbon phase, altering the properties measured? (2) Morisaki (1983, 1984) has reported that hydrophobic substrata can affect bacterial metabolism. Do such effects similarly influence adhesion to hydrocarbons? (3) Does cell–cell cooperativity play a role in BATH?

Adhesion to hydrocarbons is discussed further in Section 5.2 in the context of bacterial growth on oil.

3.1.3. Salting-out Aggregation Test (SAT)

This technique, described by Lindahl *et al.* (1981), is based on the premise that the same laws governing precipitation of protein molecules from aqueous solution hold true for aggregation of bacterial cells, i.e., that the more hydrophobic the cell, the greater its tendency to precipitate out of the solution (i.e., through cell–cell interactions) at a lower concentration of salting-out agents. In this method, bacterial cells are suspended in dilute phosphate buffer, and ammonium sulfate is added until aggregation occurs. This technique appears to correlate with other methods in most, but not all, instances (Ferreiros and Criado, 1984). Configurational changes of cell-surface structures due to the high salt concentrations may introduce errors in measurement.

3.1.4. Adhesion to Solid Surfaces

Adhesion to solid surfaces of relatively low surface free energy has been employed to test for bacterial hydrophobicity by a variety of investigators. The ability of cells from bacterial colonies to adhere directly to polystyrene has been employed as a qualititative technique for screening hydrophobic colonies (M. Rosenberg, 1981), and, more recently, as a rapid agglutination test (Lachica and Zink, 1984b). Various investigators have used adhesion to polystyrene or similar surfaces as a quantitative measure of bacterial hydrophobicity, measuring the number of adherent cells by a variety of techniques (e.g., direct counting, measurement of absorbed stain). Adhesion to solid surfaces of varying surface energies has also been studied (Gerson and Akit, 1980; Fletcher and Loeb, 1979; Dexter *et al.*, 1975; Dexter, 1979; Pringle and Fletcher, 1983). The latter authors observed that 16 freshly collected bacterial isolates adhering to plastic surfaces in freshwater exhibited the highest attachment to more hydrophobic surfaces. Absolom *et al.* (1983) have used an elegant technique (originally developed for measuring hydrophobic properties of mammalian cell surfaces) for the measurement of bacterial hydrophobicity (see Section 2). Bacteria suspended in different concentrations of

DMSO are brought into contact with smooth, solid surfaces of varying surface free energies. Using this method, these workers have been able to ascribe surface free energies to several bacteria tested. Unfortunately,the bacterial suspensions all fell within a narrow range of surface energy values; thus, it is difficult to predict whether this technique would be similarly successful with strains generally considered to be substantially more hydrophobic.

3.2. Molecular Probes

Dyar (1948), and later Hill *et al.* (1963), employed charged surfactant molecules in order to determine cell-surface hydrophobicity in staphylococcal and streptococcal cells. In such studies, small concentrations of ionic surfactant (usually negatively charged, to eliminate electrostatic interactions between positively charged probes and the net negative charge of most bacterial surfaces) were added to bacterial suspensions. Changes in the electrophoretic mobility of the cells were attributed to binding of surfactant molecules via hydrophobic interactions to the cell surface.

This principle was employed by Kjelleberg *et al.* (1980), who measured binding of radiolabeled dodecanoic acid to bacterial cells. Analysis of the results from several strains indicated that bacteria may differ in the number of binding sites available for attachment of hydrophobic molecules as well as in the affinity of such bonds. This method can yield important information concerning the nature and number of hydrophobic binding sites on the cell surface. A similar approach using palmitic acid has been proposed by Malmqvist (1983). The main concern in the use of such hydrophobic probes is their small size, with the possibility that they can intercalate within the cell envelope, thus binding to sites that are not normally exposed at the outermost cell surface.

3.3. Contact Angle Measurements (CAM)

Contact angle measurements are routinely employed by surface scientists to measure surface properties of clean, smooth, flat, dry, solid surfaces. Small drops of liquid are applied to the surface under controlled conditions and the angle of contact recorded directly. Recently, van Oss and coworkers have proposed that such measurements, conducted on bacterial layers, can provide quantitative information on the hydrophobicity of the individual cells (van Oss and Gillman, 1972; Cunningham *et al.*, 1975; van Oss, 1978), including a value for the surface energy of the bacteria measured (Absolom *et al.*, 1983). Potential drawbacks of the methodology include: (1) the cells must remain moist, but not wet; thus,

results must be obtained within a given time during the drying out process of the layer; (2) the surface, being composed of individual cells, is not flat; and (3) it is not known whether the surface presented by the outermost bacterial layer (facing upward) provides an overall picture or is the result of an alignment of cells, perhaps each projecting a patch of increased or decreased hydrophobicity. Despite these reservations, the results obtained using this method have been corroborated by other, independent techniques (Absolom *et al.*, 1983). Fletcher and Marshall (1982a) have introduced a modification (albeit, for studying substrata) in which the contact angle of air bubbles surrounded by water on the inverted cell surface is measured. This method was employed by Fattom and Shilo (1984) to study the hydrophobicity of Cyanobacteria. Similarly, Neufeld *et al.* (1980) have suggested replacing the air surrounding the water droplet by oil. Whereas most studies have employed drops of water or saline, Busscher *et al.* (1984) calculated the surface energies of oral bacteria based on contact angles of water, water–in-propanol mixtures, and α-bromonapthalene.

3.4. Two-Phase Partitioning (TPP)

Several decades ago, Albertsson (1958) proposed a method for particle separation based on partitioning between two polymeric aqueous phases that are mutually immiscible, such as dextran and polyethylene glycol. Later, this technique was used to separate bacterial cells; e.g., smooth and rough *Salmonella typhimurium* cells [reviewed in Edebo *et al.* (1980)]. Hydrophobicity of the polyethylene glycol phase can be enhanced by the covalent binding of acyl groups (Magnusson and Johansson, 1977), enhancing the affinity of hydrophobic bacteria for this phase as compared to the dextran layer. One possible problem associated with this method is that a portion of the bacteria may become trapped between the two phases (Sacks and Alderton, 1961), complicating recovery and interpretation. Nonetheless, this technique may be viewed as being relatively nondestructive, and avoids the use of high concentrations of salts or organic solvents. Gerson and Akit (1980) reported linear correlations between the TPP and CAM methods, enabling them to grade the relative hydrophobicity of several bacterial species.

3.5. Indirect Methods

Hydrophobic interactions are inhibited by a wide range of molecules, including those that exhibit surface-active and/or chaotropic activities. In some cases, a role for hydrophobic interactions in the mediation of adhesion phenomena may be invoked based on the ability of such compounds

to inhibit or reverse adhesion (Erne *et al.*, 1984; Nesbitt *et al.*, 1982). For example, adhesion of *Thiobacillus* to sulfur granules is inhibited by sodium dodecyl sulfate (Bryant *et al.*, 1984). Adhesion of *Pseudomonas aeruginosa* to erythrocytes is inhibited by chaotropic agents, but not by various sugars, suggesting the involvement of hydrophobic interactions (R. J. Doyle, personal communication). Inhibition of bacterial autoaggregation and coaggregation by detergents has underlined the importance of hydrophobicity in these phenomena (McIntire *et al.*, 1982; Stanley and Rose, 1967). Surface-active alcohols are often potent adhesion inhibitors (M. Rosenberg *et al.*, 1980a; Fletcher, 1983). E. Rosenberg *et al.* (1983a) have proposed that the majority of attached normal microbiota on the surface of human buccal epithelial cells adhere via hydrophobic interactions, since they are desorbed by low concentrations of emulsan, an amphipathic polymer.

4. Surface Components that Influence Bacterial Hydrophobicity

In preparing this section, we encountered the problem of how to denote surface components that promote bacterial hydrophobicity. To call them "adhesins" would presuppose a role in adhesion. In at least some cases, this is apparently not the case (Davison and Sanford, 1982; Kozel, 1983; Sobel and Obedeanu, 1984). We have thus elected to use the term *hydrophobins* to denote cell-surface components that contribute to cell-surface hydrophobicity; similarly, surface components that reduce cell-surface hydrophobicity have been termed *hydrophilins.*

Investigations into the hydrophobic surface properties of *Acinetobacter calcoaceticus* RAG-1 illustrate the possible complexity involved in determining the participating cell-surface components. Several lines of evidence establish a role for thin fimbriae as hydrophobins in RAG-1: (1) MR-481, a nonhydrophobic mutant of RAG-1, is deficient in thin fimbriae, (2) partial adhesion revertants possess numerous thin fimbriae, (3) shearing of fimbriae reduces hydrophobicity in RAG-1 cells grown on minimal medium, and (4) strain AB-15, an independent, thin fimbria-less mutant, is nonadherent when grown on minimal medium (M. Rosenberg *et al.*, 1982a). Thin fimbriae, however, are not the whole story, since (1) AB-15 cells are hydrophobic following growth on complex medium (despite their total lack of thin fimbriae); and (2) crossed immunoelectrophoresis of supernatants following sonic oscillation of complex-medium-grown cells reveals that the nonhydrophobic mutant MR-481 lacks at least three surface antigens, in addition to the thin fimbriae, when compared with wild-type RAG-1 cells (E. A. Bayer and M. Rosenberg, unpublished data). These results suggest that thin fimbriae are the main hydro-

phobin of RAG-1 cells grown on minimal medium, but that additional factor(s) contribute to the hydrophobicity of complex-medium-growth cells. To complicate matters further, the emulsan minicapsule of RAG-1 acts as a hydrophilin (E. Rosenberg *et al.,* 1983b). Complete loss of cell-surface emulsan leads to exposure of hydrophobins, which are apparently masked in wild-type cells (Pines and Gutnick, 1984).

In the light of such investigations, the presumption that cell-surface hydrophobicity is due to a single factor may often be premature. In some instances [e.g., *Aeromonas salmonicida* (Trust *et al.,* 1983, N. D. Parker and Munn, 1984)], bacterial hydrophobicity may indeed be due to a single hydrophobin, which dominates the outer cell surface. This, however, may be the exception rather than the rule. Bacterial hydrophobicity may often involve a delicate interplay of charged and uncharged, polar and apolar groups, exposed to varying degrees, and with varying densities over the cell surface (Kjelleberg, 1984b). Moreover, it is impossible to determine precisely where the cell surface ends and the surrounding aqueous phase begins. On a molecular level there is no sharp boundary at the outermost cell surface, and water molecules can penetrate the outer bacterial layers to varying extents. In some cases, surface projections, such as various fimbriae, fibrils, holdfasts, and so on, may themselves mediate adhesion to hydrophobic surfaces (Marshall and Cruickshank, 1973), in which case the cell itself may be tethered to the surface.

Components of the bacterial cell surface usually vary greatly as a function of growth conditions, including parameters such as aeration, temperature of growth, growth medium, and age of cells. Thus, whereas some investigators originally proposed that bacterial strains could be assigned cell-surface hydrophobicity values independent of growth conditions (van Oss and Gillman, 1972), more recent studies have shown that hydrophobic surface properties vary greatly as a function of physiological conditions (M. Rosenberg, *et al.,* 1980a, 1981; Kjelleberg *et al.,* 1980; Ofek *et al.,* 1983; Nesbitt *et al.,* 1982; Rogers *et al.,* 1984). The dramatic increase in hydrophobicity of *A. calcoaceticus* RAG-1 cells over the course of several generations of growth is illustrated in Fig. 3. Starvation effects also have a large influence on cell-surface hydrophobicity (Kjelleberg, 1984a; Kjelleberg *et al.,* 1983; Kjelleberg and Hermansson, 1984). The large differences in hydrophobicity of starved versus fed natural populations are shown in Table II. Finally, it must be emphasized that cell-surface hydrophobicity varies not only from species to species, but also from strain to strain (M. Rosenberg, 1982; Olsson and Westergren, 1982). Thus, within a given species, various combinations of hydrophobins may be responsible for the hydrophobic properties observed. Indeed, this may account for arguments such as whether the hydrophobicity of group A streptococci is mediated by lipoteichoic acid (Miorner *et al.,* 1983, 1984),

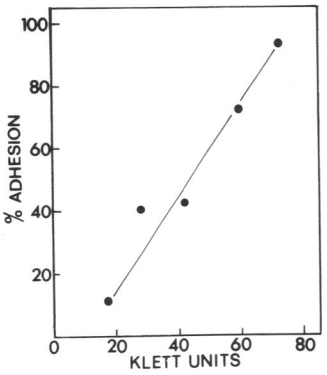

Figure 3. Adhesion to hexadecane as a function of growth. *Acinetobacter calcoaceticus* RAG-1 cells were grown in modified nutrient broth with shaking at 30°C. At various growth culture turbidities samples were removed. Cells were washed and assayed for adhesion to 0.2 ml of hexadecane as described (M. Rosenberg *et al.*, 1980a). Results are expressed as percent adhesion to hexadecane as a function of growth culture turbidity.

M protein (Tylewska *et al.*, 1979), or both (Courtney *et al.*, 1983; Ofek *et al.*, 1983; Wadstrom *et al.*, 1984); all these possibilities, and more, may be found in various strains.

The dependence of hydrophobic properties on physiological conditions can often be correlated to the synthesis, or degree of exposure, of hydrophobins and hydrophilins, as in the case of *Streptococcus pyogenes* (Ofek *et al.*, 1983). The presence of plasmids may similarly affect cell-surface hydrophobicity by allowing expression of fimbriae and other surface components (Lindahl *et al.*, 1981; Lachica and Zink, 1984a; Martinez, 1983; Ferreiros and Criado, 1984). Finally, mechanical, enzymatic, or other destructive treatments of the cell surface can alter hydrophobicity. These include studies in which buried hydrophobins are unmasked (e.g., Mackey, 1983), resulting in increased hydrophobicity, as well as those in which hydrophobins are removed, e.g., by proteolytic enzymes (Ofek *et al.*, 1983) or mechanical means (M. Rosenberg *et al.*, 1982a). The development of cell-surface hydrophobicity in *Escherichia coli* and *Sal-*

Table II. Adhesion to Octyl–Sepharose Beads and Hexadecane of the Total Microbiota of the Colon Content of Fed and Starved Flounder[a]

	Percent adhesion[b]	
Animals	To octyl–sepharose	To hexadecane
Fed	3	16
Starved	74	53

[a]P. L. Conway, J. Maki, R. Mitchell, and S. Kjelleberg (unpublished results).
[b]Percentage adhesion was measured following mixing and equilibration. Results are expressed as the percentage drop in the number of the cells remaining in the aqueous phase following the respective assays.

monella typhimurium following invasion by *Bdellovibrio* cells is an example of bacteria-inflicted damage resulting in changes in surface hydrophobicity (Cover and Rittenberg, 1984).

Several cell-surface components identified as hydrophobins in various bacteria are listed in Table III. The following sections deal with some of these examples in greater detail.

4.1. Fimbriae and Surface Fibrils

Fimbriae (or surface fibrils) have often been cited as hydrophobins. In some cases [e.g., the thick fimbriae of *Acinetobacter calcoaceticus* RAG-1 (M. Rosenberg *et al.*, 1982a)] fimbriae play no role in modulating cell-surface hydrophobicity and may even act as hydrophilins, as in the

Table III. Some Proposed Bacterial Hydrophobins

Component	Bacteria	Reference
Thin fimbriae	*Acinetobacter calcoaceticus* RAG-1	M. Rosenberg *et al.* (1982a)
M protein, lipoteichoic acid, or both	Group A streptococci	Ofek *et al.* (1983), Miorner *et al.* (1983), Wadstrom *et al.*, (1984), Tylewska *et al.* (1979)
A protein	*Staphylococcus aureus*	Jonsson and Wadstrom (1983, 1984)
"A layer" protein	*Aeromonas salmonicida*	Trust *et al.* (1983), N. D. Parker and Munn (1984)
Prodigiosin	Pigmented *Serratia* strains	Blanchard and Syzdek (1982), Hermansson *et al.* (1979), Kjelleberg *et al.* (1980)
Glucosyltransferase	*Streptococcus mutans*	D. Oakley and R. J. Doyle (unpublished results)
Outer membrane proteins	*Vibrio cholerae*	Kabir and Ali (1983)
Surface fibrils	*Streptococcus sanguis*	Gibbons *et al.* (1983)
	Yersinia enterocolitica	Lachica and Zink, (1984 a,b), Lachica *et al.* (1984)
Various fimbriae	*Escherichia coli*	Faris *et al.* (1981), Smyth *et al.* (1978), Honda *et al.* (1984), Jann *et al.* (1981), Svanborg-Eden *et al.* (1984), Ohman *et al.* (1982)
Core oligosaccharides or outer membrane lipids	Rough enterobacterial strains	Edebo *et al.* (1980), M. Rosenberg *et al.* (1982b)
Gramicidin S	*Bacillus brevis* spores	E. Rosenberg, *et al.*, 1985.

case of *Serratia* (Hermansson *et al.,* 1982). All, or almost all, of the fimbriae examined to date contain a high proportion of hydrophobic amino acid residues (Jones and Isaacson, 1983). This, however, does not necessarily imply that all fimbriae are hydrophobins, since their ability to undergo hydrophobic interactions would depend on the spatial disposition of these residues (i.e., exposure toward the bulk aqueous phase) rather than their total number. Certainly, among the fimbriae reported to act as hydrophobins, great variations are observed in the cell-surface hydrophobicity that they confer. This, in turn, may also be a function of the number of fimbriae per cell and their topographic distribution.

Of particular interest in this regard are the hydrophobic interactions attributed to mannose-sensitive ("type 1") and various mannose-resistant fimbriae of enterobacterial strains. Although fimbriated *Escherichia coli* strains are usually more hydrophobic than nonfimbriate counterparts, the degree of hydrophobicity that they confer is low. For example, the adhesion of fimbriae-bearing enterobacterial strains in HIC is largely dependent on the presence of high concentrations of salting-out agent (Jann *et al.,* 1981; Ohman *et al.,* 1982; Faris *et al.,* 1981). Moreover, whereas certain fimbriae can promote adhesion to hydrocarbons and polystyrene (M. Rosenberg *et al.,* 1982a), various fimbriated *Escherichia coli* strains do not adhere to either surface (T. Zusman and E. Ron, unpublished results; M. Rosenberg, 1982). Such observations may, however, understate the role of hydrophobic interactions (however weak) on attachment of enterobacteria to host cells, as described in Section 5.7.

The presence of fimbriae is related to bacterial adhesion in natural systems (Pertsovskaya *et al.,* 1973; Romanenko, 1979). Furthermore, it was recently found that hydrophobic fimbriae-bearing *Salmonella typhimurium* cells adhered in higher numbers to several mineral particles than nonfimbriated, less hydrophobic cells (Stenstrom and Kjelleberg, 1985).

4.2. Role of Capsule

In almost all studies [with the exception of some encapsulated staphylococci, according to Hogt *et al.* (1983a)] the presence of bacterial capsule or slime has been shown to decrease surface hydrophobicity. For example, enzymatic removal of the capsule of *Acinetobacter calcoaceticus* BD4 led to an increase in adhesion to hexadecane from 0 to 100% (E. Rosenberg *et al.,* 1983b). Cell-surface capsule reduces cell-surface hydrophobicity in a *Phormidum* sp. (Fattom and Shilo, 1984), and was reported to lower phagocytosis of *Staphylococcus aureus* by rendering the cells more hydrophilic (van Oss and Gillman, 1972). Adhesion of *Streptococcus pyogenes* to hexadecane corresponds to the absence of hyaluronic acid-containing capsule (Ofek *et al.,* 1983). A mutant of a fresh-

water *Pseudomonas fluorescens* strain producing an exopolysaccharide dominated the aqueous phase, whereas the non-exopolysaccharide-producing wild type was foam-fractionated by the adsorption bubble separation process in a fermentor (Pringle *et al.*, 1983). Recently, Runnels and Moon (1984), employing several strains of encapsulated, fimbriated *Escherichia coli* and their noncapsular mutants, showed that the presence of capsule impeded K99-mediated adhesion.

Rough mutants of smooth enterobacterial strains, such as *Salmonella typhimurium* (Edebo *et al.*, 1980), *E. coli* (M. Rosenberg *et al.*, 1980a; M. Rosenberg, 1984b), and *Proteus mirabilis* (M. Rosenberg *et al.*, 1982b), are more hydrophobic, presumably due to loss of surface oligosaccharides.

Such results suggest the antiadhesive role of capsule, and do not appear to concur with various studies that claim a major role for capsule in mediating adhesion to a wide variety of substrata (Costerton *et al.*, 1981). However, since the latter experiments are usually conducted over the course of several hours or days, the copious amounts of polysaccharide observed at attachment sites may or may not be involved in the initial attachment process. We therefore suggest that whereas capsules may act to bridge and cement layers of adhering bacteria to substrata, they are not necessarily involved in initial adhesion processes. This, however, does not apply for polysaccharide layers undergoing gel formation (Rutter, 1980).

4.3. Prodigiosin and Serratial Hydrophobicity

Prodigiosin is the cell-surface pigment that accounts for the red coloration of *Serratia* strains. Various investigators have found correlations between the appearance of prodigiosin and cell-surface hydrophobicity (M. Rosenberg *et al.*, 1980a; Kjelleberg *et al.*, 1980; Blanchard and Syzdek, 1978; Hermansson *et al.*, 1979; Syzdek, 1985), implicating this molecule as a hydrophobin in pigmented strains. However, several studies have shown that nonpigmented strains and mutants may also be substantially hydrophobic (Hermansson *et al.*, 1979; M. Rosenberg, 1984c; M. Rosenberg and Y. Blumberger, unpublished data). Moreover, a pigmented, nonhydrophobic *S. marcescens* mutant was recently isolated (M. Rosenberg, 1984c). This mutant differs from wild-type cells in the increased translucence of its colonies, suggesting that factor(s) conferring colony opacity are additional (and perhaps crucial) hydrophobins in pigmented cells, and may similarly contribute to cell-surface hydrophobicity in nonpigmented strains. In contrast to reports linking mannose-sensitive adhesins with cell-surface hydrophobicity in *E. coli* (Jann *et al.*, 1981; Ohman *et al.*, 1982), no relationship was found between mannose-sensi-

tive aggregation of yeast cells and hydrophobicity in clinical *Serratia* isolates (M. Rosenberg, and Y. Blumberger, unpublished results).

4.4. Cell-Surface Hydrophobicity in Bacterial Spores

Increased hydrophobicity of *Bacillus* spores as compared to that of vegetative cells has been shown by increased adhesion to hydrocarbon (M. Rosenberg, 1984a; Doyle *et al.*, 1984) and partitioning in an aqueous two-phase system (Sacks and Alderton, 1961) and at the air–water interface (Boyles and Lincoln, 1958; Gaudin *et. al.*, 1960). Cell-surface hydrophobicity in spores may provide a biological advantage in enabling their dispersal via aerosols (see also Section 5.3). However, increased hydrophobicity of spore versus vegetative cells is not universal: Kupfer and Zusman (1984) have recently reported that sporulating *Myxococcus xanthus* cells become less and less hydrophobic as sporulation proceeds.

A possible role for the antibiotic gramicidin S in mediating the hydrophobicity of *B. brevis* spores has been recently demonstrated (E. Rosenberg *et al.*). Whereas wild-type spores, which produce the antibiotic, were hydrophobic, gramicidin S-negative mutant spores were not; however, extraneous addition of gramicidin S increased the hydrophobicity of the mutant spores in a dose-dependent manner. These data suggest that the cationic moiety of gramicidin S binds to anionic receptors on the spore surface, exposing the hydrophobic amino acid residues of the antibiotic molecule on the cell surface.

5. Hydrophobicity and Adhesion in the Environment and Host

5.1. Introduction

In the present section we deal with the role of hydrophobic interactions in bacterial adhesion to a variety of surfaces in the open environment as well as in the host. Whereas most *in vitro* investigations deal with adhesion of washed bacterial suspensions to clean surfaces, the *in situ* situation is usually quite different. Interfaces in aqueous surroundings are rapidly coated with a "conditioning" layer of adsorbed macromolecules and lipids (Fletcher, 1976); Fletcher and Marshall (1982a) proposed a useful method for *in vitro* measurement of the interfacial properties of such films, which may profoundly affect bacterial adhesion (Kjelleberg and Stenstrom, 1980). Morever, bacteria (particularly within animal hosts) adsorb, whether specifically or not (Miorner *et al.*, 1980; Absolom *et al.*, 1982), antibodies and other macromolecules (Wadstrom *et al.*,

1984). This, in turn, either promotes or inhibits the ability of the cell to attach, depending on the cell-surface hydrophobicity of the cell prior to adsorption and the nature of the adsorbing molecules (van Oss, 1978). The *in situ* coating of the bacterial surface and the attachment substratum is generally difficult to estimate, making it hard to extrapolate from laboratory experiments to *in situ* adhesion. Several strategies in this direction would include the following: (1) Comparision of the ability of laboratory strains to adhere to the actual surface under study as a function of their relative hydrophobic surface properties. One such study by Svanberg *et al.* (1984) demonstrated that hydrophobic *Streptococcus mutans* is better able to attach to and colonize oral surfaces of human volunteers as compared to a nonhydrophobic variant; (2) attempting to desorb attached bacteria by means of agents that interfere with the mechanism under study; and (3) direct examination of the adherent properties of bacteria isolated directly from the niche under study. This last point is of great importance in the light of experiments showing that cell-surface hydrophobicity may be dependent on the degree of starvation of cells (Kjelleberg and Hermansson, 1984), strain history (Westergren and Olsson, 1983), and other factors that differ widely between cells in the environment and in the test tube. With few exceptions (e.g., M. Rosenberg *et al.*, 1983c), the cell-surface hydrophobicity of bacteria taken directly from nature and tested without further growth has not been investigated. This is unfortunate, since such experiments are not difficult to perform (some results on hydrophobicity of bacteria taken directly from gastrointestinal systems appear in Table II and in Section 5.6).

5.1.1. "Specific" versus "Nonspecific" Hydrophobicity

Various investigations attest to the nonspecific nature of bacterial adhesion to low-energy surfaces. For example, *A. calcoaceticus* RAG-1 adheres to liquid and solid hydrocarbons, plastic surfaces, human teeth, buccal epithelial cells and air bubbles, exposed metal surfaces, and so on (M. Rosenberg, 1982, and unpublished observations). Thus, it is not surprising that many researchers agree that hydrophobic interactions that mediate bacterial adhesion are not dependent on molecular complementarity between bacterial surface components and receptors on the attachment substratum or on the "molecular texture" of the two surfaces. However, the possibility has been raised that in adhesion to host tissue, specific recognition mechanisms between hydrophobic moieties and stereospecific receptors do occur (Ofek *et al.*, 1983; Simpson *et al.*, 1980), much in the same way that serum albumin binds hydrophobic molecules. Thus, in the case of adhesion of *S. pyogenes* to buccal epithelial cells, specific bacterial hydrophobins (e.g., M protein–lipoteichoic acid com-

plex) would be recognized by epithelial cell receptor(s) (e.g., fibronectin) and adhesion mediated by this specific mechanism (Abraham *et al.*, 1983; Courtney *et al.*, 1983). It is also possible that hydrophobic interactions, in addition to their ability to mediate adhesion *per se*, may also contribute to stereospecific adhesion mechanisms. In the case of mannose-sensitive adhesion, for example, great importance may be attributed to apolar moieties in the immediate proximity of the mannose-binding site. Firon *et al.* (1982) have reported that mannosides of an increasingly hydrophobic nature possess much higher affinity for the binding site, similar to results obtained with the lectin concanavalin A (Lis and Sharon, 1977). Similarly, the presence of mannose has been reported to decrease surface hydrophobicity of *E. coli* bearing type 1 fimbriae, presumably due to some conformational change at the sugar-binding site (Ohman, 1983). Such data suggest that neighboring hydrophobic interactions may promote mannose-sensitive binding in *E. coli* strains.

5.2. Adhesion and Growth at the Solid–Liquid Interface

The possible role of hydrophobicity and adhesion in the life cycle and survival of bacteria in the environment is discussed in the present section.

Adhesion as a tactic in starvation survival was proposed by Dawson *et al.* (1981). More recently, the existence of an increased degree of irreversible binding and hydrophobicity upon starvation has been demonstrated for some bacterial species (Kjelleberg and Hermansson, 1984); in other bacteria tested, this trend was not observed (Kjelleberg *et al.*, 1985a). Other researchers (Ellwood *et al.*, 1982), studying a diverse microbial community, showed a correlation between carbon limitation and attachment. In this context, it should be emphasized that each organism has its own specific starvation-survival pattern (R. Y. Morita and R. Jones, personal communication).

The role of interfaces as areas of nutrient supply in nutrient-deprived ecosystems has been highlighted (Marshall, 1979). The question of whether bacterial activity at such surfaces is affected by the surface *per se*, by nutrients at the interface, or both, is currently under consideration by various investigators (e.g., Morisaki, 1983, 1984; Jeffrey and Paul, 1985; Fletcher, 1985; Gordon and Millero, 1985). It is noteworthy, however, that the scavenging of surface-localized long-chain fatty acids was favored by a hydrophobic *S. marcescens* strain as compared with its hydrophilic mutant (Kefford *et al.*, 1982). These authors also showed that reversibly bound bacteria, attaching and rapidly desorbing, scavenge surface-localized material. This utilization of surface-immobilized substrates has more recently been shown to be a common feature for a wide

range of natural marine isolates (Hermansson and Marshall, 1985); whereas irreversibly bound cells exhibited more efficient utilization, reversibly bound bacteria, which are prevalent in the environment, were also able to utilize surface-immobilized materials.

Based on several investigations (e.g., Dawson *et al.,* 1981; Kjelleberg *et al.,* 1982; Kjelleberg and Hermansson, 1984; Hermansson and Dahlback, 1983; Pedros-Alio and Brock, 1983), Dahlback (1983) suggested a life cycle in which planktonic, starved hydrophobic bacteria adhere readily to available interfaces, efficiently scavenge surface-localized materials, increase in biomass and size, and then leave the interface, usually in the form of a daughter cell following division (Kjelleberg *et al.,* 1982) to face the nutrient-deprived conditions in the planktonic phase. The mechanism for bacterial desorption from the interface may involve changes in cell-surface hydrophobicity or, alternatively, localized bacterial production of amphipathic materials that remove bound cells (E. Rosenberg *et al.,* 1983a).

5.3. Adhesion and Growth at the Oil–Water Interface

Adhesion of bacteria and yeast to hydrocarbons has often been observed by microbiologists interested in the field of oil degradation, but such investigators considered this property to be specific to oil-degrading microorganisms (Kennedy *et al.,* 1975; Miura *et al.,* 1977), ignoring several previous investigations (Mudd and Mudd, 1924a; Marshall and Cruickshank, 1973). M. Rosenberg *et al.* (1980a,b) showed that oil-degrading bacteria may or may not adhere to hydrocarbons, and that nondegraders (e.g. *Serratia marcescens, Staphylococcus aureus*) may adhere in high proportions despite their inability to metabolize hydrocarbon. These results suggested the use of bacterial adhesion to liquid hydrocarbons as a general technique for determining cell-surface hydrophobicity (Section 3.1.2) (M. Rosenberg *et al.,* 1980a,b.) Another interesting result of this study was that adhesion of *Acinetobacter calcoaceticus* RAG-1 is not limited to hydrocarbons that it metabolizes. Later studies (M. Rosenberg and Rosenberg, 1981; M. Rosenberg *et al.,* 1982a) addressed the possible role of adhesion in enabling growth of RAG-1 on hexadecane. RAG-1 cells grew readily on hexadecane under conditions of limited hydrocarbon emulsification (low initial cell density and mild agitation), whereas its nonadherent mutant, MR-481, did not grow unless emulsifier was added. Moreover, prolonged incubation of MR-481 cells in hexadecane growth medium led to the eventual growth of increasingly adherent revertants (M. Rosenberg *et al.,* 1982a; Pines and Gutnick, 1984). In a comparison of the adhesion of RAG-1 with that of an oil-degrading strain of *Pseudomonas aeruginosa,* the latter strain exhibited

extremely poor affinity for hydrocarbons, even following growth on hexadecane (M. Rosenberg *et al.*, 1980b; Nakahara *et al.*, 1981). These results suggest that nonadherent strains of bacteria that grow on hydrocarbons in the laboratory (i.e., in closed systems that enable emulsification of the insoluble substrate) will not grow well on oil *in situ*. In the open environment, adhesion to oil is frequently observed (e.g., Wyndham and Costerton, 1982); however, emulsification of oil droplets in the open waters would allow them to diffuse away from the cells. Some investigators (working primarily with *Pseudomonas* strains) have argued that adhesion is not an important factor in growth on oil (Reddy *et al.*, 1982; Chakravarty *et al.*, 1975). This may well be true in the case of growth on aromatic hydrocarbons, which are generally more water-soluble and more toxic than paraffins.

An interesting aspect of growth on hydrocarbons is the relationship between bound and free cells. Kirschner-Zilber *et al.* (1980) studied adhesion and growth of a marine pseudomonad on solid *n*-tetracosane; the investigators proposed that individual cells could alternate between the water–paraffin interface and the bulk aqueous phase, with a continual flux of adhering and desorbing cells. The appearance of free *A. calcoaceticus* RAG-1 cells during growth on hexadecane is not due to their inability to adhere to hexadecane, but probably results from their desorption by emulsan, the amphipathic polysaccharide released by the cells (M. Rosenberg and Rosenberg, 1981; E. Rosenberg *et al.*, 1983a). It has been shown (M. Rosenberg, 1982) that bacteria adhering to hexadecane can be desorbed following freezing and thawing of the hydrocarbon phase (Fig. 4). This phenomenon may reflect a basic difference between adhesion at deformable (liquid–liquid and liquid–gas) and relatively nondeformable (liquid–solid) surfaces (P. Rutter, personal communication). Assuming that bacterial adhesion at the oil–water phase does entail gross deformation of the interface [as proposed by Mudd and Mudd (1924b)], the phase transition from liquid to solid hexadecane may reduce the surface available for interaction and thus decrease the energetic favorability of adhesion, with the resultant desorption of cells. The observation that oils readily remove bound bacteria from polystyrene (M. Rosenberg *et al.*, 1983b) may be related to preferential partitioning of bacteria at liquid–liquid rather than liquid–solid interfaces.

Another interesting observation is that bacteria often tend to adhere to oil droplets in patches, both during growth on oil (E. Rosenberg and M. Rosenberg, unpublished data) and following the adhesion assay. Such patches may arise from cell–cell interactions at the interface. Further evidence for this possibility is that bacteria desorbed from hexadecane following freezing and thawing of the hydrocarbon phase are recovered in the bulk aqueous phase as aggregates, rather than individual cells (M. Rosenberg, 1982).

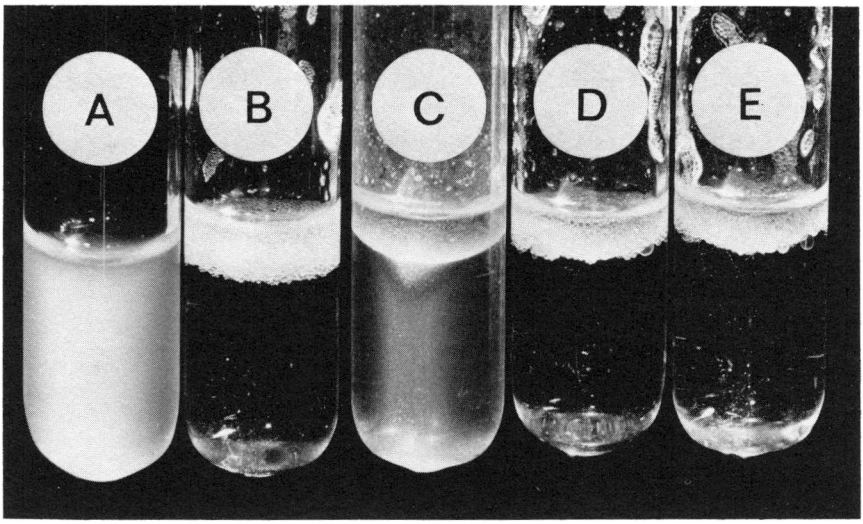

Figure 4. Release of cells bound to hexadecane due to phase transition of hydrocarbon. RAG-1 cells were tested for adhesion to hexadecane (tubes B and C) and octane (tubes D and E) as previously described (M. Rosenberg *et al.*, 1980a). Following the adhesion assay, test tubes C and E were cooled to 12°C for 3 min, then allowed to equilibrate with room temperature (22°C). In the tube containing RAG-1 cells bound to hexadecane (tube C), the hexadecane droplets solidified, and following equilibration at room temperature, melted and coalesced, with concomitant release of clumped RAG-1 cells back into the aqueous phase. No such effect was observed in a similarly treated tube (tube E) containing cells bound to octane (which did not undergo phase transition). Test tube A contains the washed bacterial suspension prior to the adhesion assay. [From M. Rosenberg (1982).]

5.4. Adhesion at Gas–Liquid Interfaces

Mechanisms for adhesion at gas–liquid interfaces have been recently reviewed (Kjelleberg, 1984b, 1985). It appears that cell-surface hydrophobicity is the prime (Hermansson *et al.*, 1982), although not the sole (Kjelleberg and Stenstrom, 1980), criterion in determining attachment at the air–water interface under conditions simulating those present in the environment. A large number of bacteria adhering to the gas–liquid interface of marine waters were tested and found to be significantly more hydrophobic than free-living bulk water bacteria (Dahlback *et al.*, 1980, 1981). Gas–liquid interfaces are not confined in nature to the surface microlayers of oceans and lakes (Norkrans, 1980), but include bubbles rising through the water column and aerosol production by bursting bubbles (Blanchard and Syzdek, 1978, 1982; Bezdek and Carlucci, 1974; B. C. Parker *et al.*, 1983; Wendt *et al.*, 1980; Weber *et al.*, 1983). The significance of such aerosols as microbial carriers has long been recognized (e.g.,

Woodcock, 1948). Not all bacteria are aerosolized with equal efficiency (Hejkal *et al.*, 1980); mediating factors include the sizes of both cell and bubble, and the cell-surface hydrophobicity (Blanchard, 1983). In the laboratory, adhesion at the air–water interface has proven an efficient means of separating bacteria (Boyles and Lincoln, 1958; Gaudin *et al.*, 1960; Rubin *et al.*, 1966), viruses (Morrow, 1969), and yeast (Ouchi and Akiyama, 1971; Iimura *et al.*, 1980) based on relative hydrophobicity. The latter investigators showed correlations between hydrophobicity and surface pellicle formations. Neufeld and Zajic (1984) have recently demonstrated that partitioning of washed suspensions of *A. calcoaceticus* cells at the air–water interface is accompanied by a significant decrease in surface tension. This finding may lead to an additional means for measuring bacterial hydrophobicity.

5.5. Life Style of Cyanobacteria

Fattom and Shilo (1984) have demonstrated a striking correlation between the mode of growth of aquatic cyanobacteria and their ability to adhere to hydrocarbon. Whereas benthic strains, which grow attached to submerged solid surfaces (sediments, stones, plant surfaces), adhered to hexadecane, free-floating (planktonic) types were invariably nonadherent. The hydrophobic surface properties of benthic cyanobacteria were further demonstrated using other techniques for studying cell-surface hydrophobicity: bubble contact angles on cell layers and adhesion to phenyl–Sepharose beads and to polystyrene. Adhesion of benthic cyanobacteria to hexadecane was dependent on the presence of divalent cations in the assay buffer. Fattom and Shilo also isolated mutants of the hydrophilic planktonic strain *Spirulina platensis* that exhibited increased hydrophobicity. Of particular interest are induced changes in hydrophobicity in *Phormidium* that can be correlated ultrastructually to changes in the cell surface (Y. Bar-Or, M. Kessel, and M. Shilo, in preparation).

5.6. Role of Hydrophobic Interactions in the Oral Cavity

The oral cavity has several advantages over other host sites in the study of adhesion phenomena: (1) it is more readily accessible than most other host surfaces; (2) it is usually very heavily colonized with an extremely wide range of adherent bacteria; and (3) since the majority of oral microbiota do not flourish outside the oral cavity, the crucial role of adhesion is patently self-evident (Gibbons, 1980).

Microscopic observation of the adherent normal microbiota of the human mouth reveals that each oral surface is colonized by a different

pattern of bacteria. For some time, it was believed that adhesion in the oral cavity was mediated by specific lectin-like recognition mechanisms (Gibbons, 1980) and that such specific adhesion correlated to colonization patterns (Gibbons *et al.,* 1976). However, there has been little success in demonstrating such mechanisms [one exception is the lactose-specific coaggregation of oral bacteria (McIntire *et al.,* 1982]. During the past 4 years, the possibility that nonspecific hydrophobic interactions play a major role in oral adhesion has been subjected to extensive experimentation. Some of the main results may be summarized as follows: (1) Oral bacteria are predominantly hydrophobic, including fresh oral isolates from the tooth surface (Weiss *et al.,* 1982), cariogenic laboratory strains (Nesbitt *et al.,* 1982, Olsson and Westergren, 1982; Gibbons and Etherden, 1983), and plaque bacteria obtained and tested directly from the tooth surface (M. Rosenberg *et al.,* 1983c). Some instances in which pronounced cell-surface hydrophobicity was not observed may be due to excessive strain transfer (Westergren and Olsson, 1983) or choice of growth conditions (Rogers *et al.,* 1984). (2) Adhesion to the model tooth surface made up of saliva-coated hydroxyapatite (s-HA) correlates with cell-surface hydrophobicity in various oral strains tested (Nesbitt *et al.,* 1982; Gibbons and Etherden, 1983; Westergren and Olsson, 1983. (3) The inability of nonhydrophobic mutants to adhere to hydrophobic test surfaces, such as hydrocarbons or polystyrene, correlates with their inability to adhere to human teeth (Weiss *et al.,* 1985) or s-HA (Gibbons *et al.,* 1983) compared to the adherent wild-type cells. (4) A hydrophobic fresh isolate of *S. mutans* is retained in the human oral cavity, whereas a nonhydrophobic variant is not (Svanberg *et al.,* 1984). Moreover, the hydrophobic strain proved more cariogenic in laboratory animals than the nonhydrophobic variant (J. Olsson, personal communication). (5) Emulsan, a bacterial amphiphile which blocks adhesion to hydrocarbons, similarly inhibits adhesion of oral and nonoral strains to human buccal epithelial cells and desorbs most of the adherent oral microbiota as well (E. Rosenberg *et al.,* 1983a). (6) Bacteria obtained directly from dental plaque adhere to human buccal epithelial cells *in vitro;* this adhesion is inhibited by the surfactants sodium dodecyl sulfate and Tween 80 (M. Tal, E. Weiss, and M. Rosenberg, unpublished data).

The critical role that saliva probably plays in influencing oral adhesion via hydrophobic interactions is a complex one. On a thermodynamic level, the surface tension of saliva would be expected to influence adhesion to all oral surfaces bathed by it (Busscher *et al.,* 1984). On a molecular level, salivary glycoproteins, antibodies, and other agglutinins can coat the bacterial surface, the substratum, or both, thus affecting adhesion; varying surface tensions and concentrations of such components from subject to subject complicate matters further (Beighton, 1984;

Abbott and Hayes, 1984). In light of such complexities, simpler model systems may prove preferable to s-HA in understanding saliva-mediated effects.

5.7. Adhesion to Other Host Surfaces

Much investigation into adhesion to host tissue has been directed at the gastrointestinal (GI) tract. As in the case of the oral cavity, there is ample evidence to suggest that hydrophobic interactions play a greater role in adhesion than is generally recognized: (1) Certain surfaces in the GI tract are extremely hydrophobic (Hills *et al.,* 1983); moreover, the mucus layer coating intestinal surfaces possesses hydrophobic properties (Smith and LaMont, 1984). (2) A large proportion of hydrophobic bacteria can be found *in situ,* in human fecal samples (Fig. 5) and in the flounder gut (Table II). (3) Adhesion of rough enterobacterial strains to intestinal mucosal cells in greater than that of less hydrophobic, smooth, wild-type cells (Perers *et al.,* 1977; Edebo *et al.,* 1980). Similarly, adhesion of enteropathogenic *Escherichia coli* strains correlates with increased fimbria-mediated hydrophobicity (Smyth *et al.,* 1978; Lindahl *et al.,* 1981; Faris *et al.,* 1981), although there is much disagreement over which organisms are the more hydrophobic (Jann *et al.,* 1981; Ohman *et al.,* 1982; Lindahl *et al.,* 1981). (4) Many laboratory strains of normal intestinal microbiota as well as pathogens that may be associated with the GI tract exhibit cell-surface hydrophobicity. These include strains of *Yersinia enterocolitica* (Faris *et al.,* 1983; Martinez, 1983; Lachica and Zink, 1984a,b; Lachica *et al.,* 1984), *Vibrio cholerae* (Faris *et al.,* 1982; Kabir and Ali, 1983), *Salmonella* (Jiwa and Mansson, 1981; Xiu *et al.,* 1983), *Bifidobacterium* (H. J. M. Oop den Camp, A. Oosterhof, and J. H. Veerkamp, unpublished data), and *Shigella* (Edebo *et al.,* 1983).

Cell-surface hydrophobicity has been reported in a variety of potentially pathogenic bacteria, including *Legionella pneumophila* (Bohach and Snyder, 1983), *Staphylococcus aureus* (M. Rosenberg *et al.,* 1980a; Jonsson and Wadstrom, 1983, 1984; Malmqvist, 1983), *Streptococcus pyogenes* (M. Rosenberg *et al.,* 1981; Ofek *et al.,* 1983; Miorner *et al.,* 1983, 1984; Tylewska *et al.,* 1979), *Mycobacterium lepraemurium* (Katoh and Matsuo, 1983), *Neisseria gonorrhoeae* (Magnusson *et al.,* 1979a,b), and *Chlamydia psittaci* (Vance and Hatch, 1980).

The adhesion of *Lactobacillus fermentum* to the nonsecreting, keratinized part of the stomach in mice was recently shown to correlate with cell-surface hydrophobicity; the presence of erythrosine in the bacterial growth medium reduced hydrophobicity and abolished adhesion to the mouse stomach tissue (P. O. Conway and R. F. Adams, unpublished results).

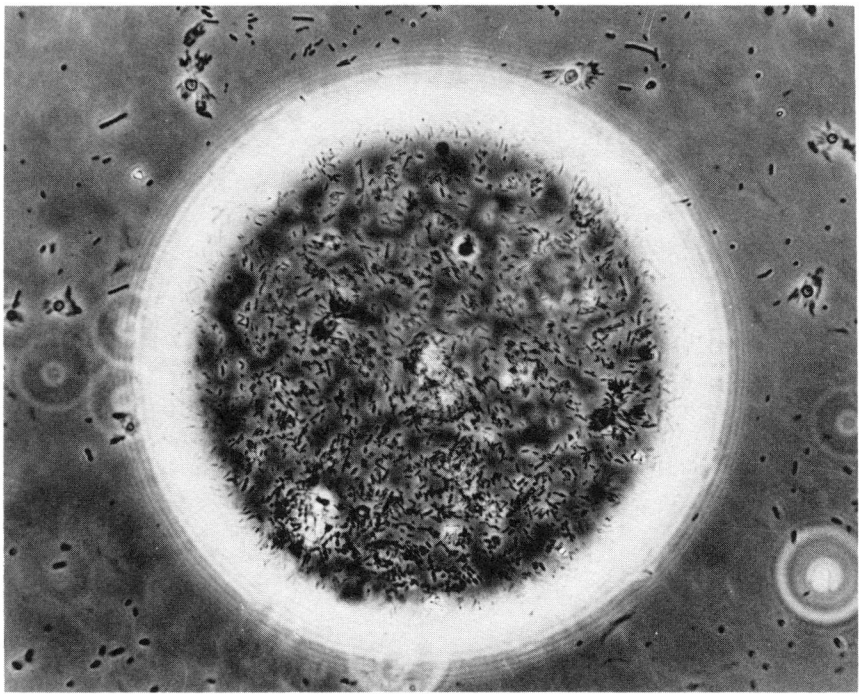

Figure 5. A fresh sample of human feces was washed and suspended in 150 mM phosphate buffer, pH 7.1, to an optical density of 0.9 at 400 nm. Adhesion to 0.2 ml of *n*-hexadecane, *n*-octane, and *p*-xylene was performed as previously described (M. Rosenberg *et al.*, 1983a). Following the mixing, the bottom aqueous phase (1.2 ml) was removed to fresh tubes containing 0.2 ml of the corresponding hydrocarbon, and the phases again mixed. Percent adhesion, as calculated from the drop in turbidity, was: hexadecane, 17%; octane, 45%; xylene, 49%. Shown is an octane droplet with adherent cells, surrounded by aqueous phase, following the mixing procedure. In general, the smaller bacteria tended to adhere at the interface, whereas the larger rods remained in the bulk aqueous phase.

The role of hydrophobic interactions in bacterial adhesion to phagocytic cells has been widely studied in recent years (van Oss, 1978; Absolom *et al.*, 1981, 1982; Edebo *et al.*, 1980); the extent of its participation in relation to more specific mechanisms, however, remains a point of controversy. Hydrophobic interactions play a role in adhesion of *Mycoplasma* to erythrocytes (Banai *et al.*, 1978, 1980; Sobeslavsky *et al.*, 1968), and have probably been overlooked in many cases of bacteria-mediated hemagglutination (R. J. Doyle, personal communication). Similarly, despite the obviously hydrophobic surface properties of human skin and hair, we are not aware of extensive studies on the role of hydrophobic interactions in mediating bacterial adhesion to such surfaces. Do hydro-

phobic pulmonary surfactants (Cotton and Hills, 1984) mediate or inhibit adhesion to lung tissues? Similarly, what is the role of hydrophobic interactions in mediating adhesion to aging or damaged cells and tissues, e.g., cornea? Is hydrophobicity a factor in adhesion to ear tissue (Hills, 1984a)?

The increasing use of polymeric implants and catheters has led various researchers to study bacterial adhesion to such materials. In many cases, hydrophobic interactions appear to be an important factor (Ashkenazi and Mirelman, 1984; Sugarman, 1982; Hogt *et al.*, 1982, 1983b).

In view of the above, the role of hydrophobic interactions in bacterial pathogenesis is twofold. Cell-surface hydrophobicity may serve to enable initial attachment of bacteria, but may also aid in their removal by phagocytic cells. Ofek *et al.* (1983) have suggested that pathogens may alter surface hydrophobicity as a function of the stage of infection; *S. pyogenes* cells, initially hydrophobic, adhere to and then colonize epithelial cells, but following invasion may become hydrophilic and nonadherent through the elaboration of hyaluronic acid capsule. The observation (Y. Blumberger and M. Rosenberg, unpublished data) that clinical *Serratia* strains become hydrophilic when grown above 37°C is suggestive of a similar example. This phenomenon bears some resemblance to adhesion and desorption of bacteria at marine interfaces (section 5.2).

6. Conclusions

Bacterial adhesion is a multifaceted subject. In some cases (e.g., adhesion of normal human microbiota, cell immobilization, degradation of insoluble pollutants, phagocytosis, nitrogen fixation), adhesion can benefit man. In other situations (colonization of host tissues by pathogens, biofouling) we seek the means to control, inhibit, and desorb adherent microorganisms. Whether trying to promote or prevent bacterial adhesion, an understanding of the underlying mechanisms is of prime importance.

In the present review we have looked at several adhesion phenomena in which hydrophobic interactions appear to play a central role. The possibility exists that the role of hydrophobic interactions in bacterial adhesion is far wider than is generally appreciated. In fact, the participation of hydrophobic interactions in adhesion phenomena is often overlooked, particularly in studies searching for specific (usually sugar-inhibited) adhesion mechanisms. As Kauss and Young (1983) have pointed out, in many investigations a battery of sugars is tested for their ability to inhibit the observed adhesion (if none work, it is often assumed that more exhaustive attempts will eventually uncover the one that does). Only

rarely have inhibitors of hydrophobic interactions been compared alongside sugars for their effect on adhesion (e.g., McIntire *et al.,* 1982). Another case in point is the inhibition of adhesion by cell-surface components. It is generally held that the ability of a cell-surface component to inhibit adhesion demonstrates its role as an adhesin in the phenomenon studied. But what if the component is itself an amphipathic molecule, capable of blocking hydrophobic interactions between cell and surface, such as lipopolysaccharide or lipoteichoic acid (Carruthers and Kabat, 1983; Botta, 1981)? In such instances inhibition of adhesion may reflect the participation of hydrophobic interactions rather than a role for the component *per se.* Our appreciation of the extent to which hydrophobic interactions mediate various bacterial adhesion phenomena could be greatly augmented by including agents that interfere with hydrophobic interactions (e.g., surfactants, chaotropic agents) in adhesion assays.

If hydrophobic interactions are, as we submit, chiefly responsible for a great many adhesion phenomena, several corollaries arise. First is the distinct possibility that many bacteria that adhere to surfaces are not too particular about the substrata to which they stick. It can then be argued that the small percentage of cells that do attach to a hospitable surface can quickly proliferate into colonies far outnumbering those that have gone astray. If this is the case, then we must remember when scrutinizing a natural surface that not all the adherent cells we observe may have "meant" to adhere. Similarly, the relative numbers of microorganisms found on a given surface say little about their relative adhesion ability: *A. calcoacaceticus* RAG-1, for example, adheres in much higher numbers to human buccal epithelial cells than does *S. pyogenes,* despite the inability of the former to colonize the oral mucosa (M. Rosenberg *et al.,* 1981; E. Rosenberg *et al.,* 1983a). Similarly, the low relative numbers of filamentous bacteria on buccal epithelial cells as compared with their predominance on the tooth surface (Gibbons, 1980) may be primarily related to their subsequent colonizing ability rather than their initial adhesion. Washed suspensions of filamentous dental plaque bacteria do adhere to buccal epithelial cells *in vitro* in the absence of saliva (M. Tal, E. Weiss, and M. Rosenberg, unpublished results).

Future investigations will be required to deal with the likelihood that the cell-surface hydrophobicity of bacteria *in situ* differs greatly from results obtained using laboratory strains: (1) laboratory strains may lose hydrophobic surface properties upon subculturing; moreover, adherent and aggregating strains, being more difficult to work with in the laboratory, may, over the course of years, have been discarded in favor of less problematic, nonadherent clones; (2) cells in the natural environment may adsorb proteins and other polymers from the surroundings, which in turn affect their surface properties; and (3) bacteria in the environment

are often starved; starvation conditions generally result in an increase in hydrophobicity and adhesion.

A problem of increasing interest is the manner in which bacteria modulate cell-surface hydrophobicity in order to detach from, as well as attach to, surfaces. It is clear that bacteria may detach from interfaces as well as adhere to them both in the open environment and in the host. It would be of great interest to learn whether such microorganisms respond to subtle changes in their natural environment which signal them to alter cell-surface hydrophobicity accordingly; in the test tube such changes often appear merely to be related to phase of growth.

How does bacterial hydrophobicity affect other physiological functions? For example, cell-surface hydrophobicity has often been related to the absence of capsule; however, since bacterial capsules may serve to protect the cell from injurious external factors (desiccation, toxic compounds, etc.), the absence of capsule may put the cell at some disadvantage. It has been suggested that cell-surface hydrophobicity is correlated with increased sensitivity to hydrophobic compounds and antibiotics (Miller, 1983; Roantree *et al.,* 1977; Mackey, 1983). However, no correlations between cell-surface hydrophobicity and sensitivity to hydrophobic antibiotics were observed in *S. typhimurium* (Kjelleberg *et al.,* 1985b) or in *A. calcoaceticus* RAG-1 (M. Rosenberg, unpublished data), suggesting that the proposed changes are related to permeability and degree of exposure of outer membrane constituents (Roantree *et al.,* 1977; Leive *et al.,* 1984).

Despite the many techniques available for measuring bacterial hydrophobicity, it is probable that we shall see novel and improved methodology in coming years. Hopefully, these will aid researchers in their attempts to answer the many outstanding questions in this field. One of the most exciting prospects is that the novel genetic techniques that have proven so successful in the study of several *Escherichia coli* adhesion systems will be extended to adhesion phenomena of the type described in this review; some indications of this are already apparent (e.g., Martinez, 1983; Lachica and Zink, 1984a,b; Lachica *et al.,* 1984) in studies on plasmid-mediated hydrophobicity in *Yersinia enterocolitica.*

In closing, we would like to mention another type of close approach that has been mediated by hydrophobic interactions. Ecologically and medically oriented adhesion researchers, once separated by an abyss of different interests and outlooks, have been drawn together by this common mechanism of bacterial adhesion. Hopefully, the synergism resulting from this merger, already evident in many of the investigations we have discussed here, has only just begun.

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